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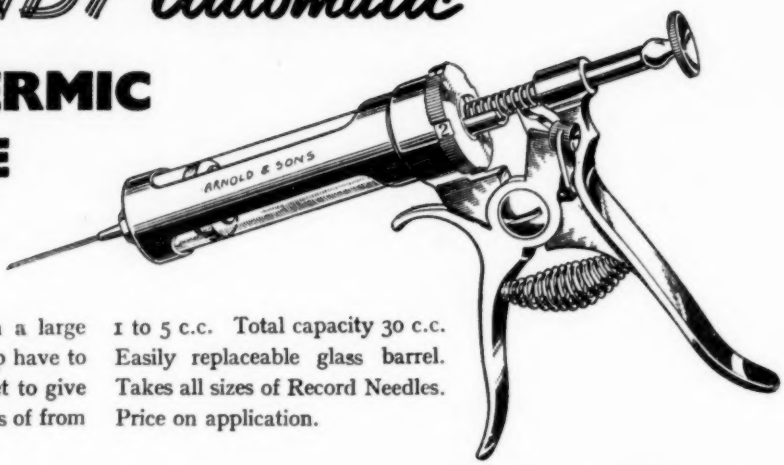

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The Approach to the Herd Infertility Problem*

BY

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Ministry of Agriculture, Fisheries and Food, Cambridge

A MODIFICATION of Hignett's (1949) definition of a problem herd, that is, one-third to have required more than three services, or to have lost equivalent time, is accepted as of most practical use. However, 24 per cent. of requests for specialist assistance by the writer related to herds in which on the above basis the breeding performance fell within normal limits. Advance application for the history helps to minimise such resulting misuse of working time. By averaging such regional figures for herd problem incidence as are available, and on a basic assumption of 20 head as the countrywide mean herd size, it appears that herd breeding problems may involve 5,000 herds and 40,000 to 70,000 infertile cows in Great Britain annually. Not more than 50 specialists who engage in this work, of whom about 40 per cent. are employed by the Ministry, and about 20 per cent. each by the Milk Marketing Board, other A.I. organisations, and by private firms, appear to be able to investigate little over half this number of problems, leaving the remainder to the general practitioner. This incidence of herd breeding problems contrasts with the loss to slaughter from *permanent infertility*, which can be estimated with some approach to accuracy at 200,000 cows per annum, and is thus economically much more important, although tending to attract less attention.

It is useful to separate herd problems associated with *specific infection* from the others, even though some functional element often complicates such outbreaks, Leptospirosis, trichomoniasis, mycosis and pleuro-pneumonia-like organism infection seem to play no part or only small parts in Great Britain. Virus infertility transmitted by the bull is recognised in Denmark, South Africa and the U.S.A.; there is growing acceptance that it may be important here. Brucellosis, regarded less as a cause of abortion than as a venereal infertility-producing agent, was involved in about 20 per cent. of the infectious problems encountered by nine specialists working in widely scattered areas. Attention is drawn to Munro's report (1957) of transmission through A.I. from a blood-negative bull, and to the infection of Strain 19-vaccinated cattle by the venereal route. Terpstra (1956) successfully produced a syndrome similar to the field-encountered disease experimentally, by intra-cervical Strain 19 vaccination; this appears to give a satisfactory general immunity, with a negative blood titre, after the expiry of the initial infertility period. The extension of the use of this route and adoption of Bendixen & Blom's (1947) sperm-agglutination test may assist in control. Nutrition levels may affect the duration and degree of immunity conferred by vaccination.

The incidence of vibriosis as reported varies from 40 to 17 per cent. of problems investigated; this incidence may be falling, and may always have been lower than the mean in the Eastern Counties. In application of the mucus agglutination test, samples from 6 animals make a suggested herd-minimum; strong positive and suspicious reactions totalled respectively 24 and 21 per cent, in an extensive series of test results from herds which proved positive, personally studied; contrasted with 5 per cent. suspicious (residue from former infections?) in the negative herds. The significance, incidence (relatively low), and differentiation (possible on herd test basis) of false positive reactions, possibly attributable to the H_2S-positive *Vibrio* described by Terpstra (1956), require further research. Rasbech's report (1955) of a significant proportion of failures, in attempts to treat bulls with antibiotic parenterally and by sheath irrigation, may merit wider attention than it has sometimes recently received. There is evidence that vibriosis uncomplicated by the synergistic effect of a functional component, an event probably of common occurrence,

* Summarised from a paper presented to the Southern Counties Veterinary Society, May 1st, 1957.

is a relatively mild disease. Such evidence includes Hignett's (1954) demonstration of increased evidence of pathogenic effects of organisms when excess phosphate is fed ; Heinemann's (1956) and Terpstra's association of the disease with abnormal soil phosphate levels and ovarian cyst production, etc.—this latter of proven genetic/functional origin ; the occurrence of agglutination-positive animals in normal breeding herds (Lawson) ; and the failure of a proportion of positive herds, where infection appeared endogenous, to respond to antibiotic treatment (Heath, 1954).

Herd functional infertility is more difficult to deal with than the infectious type, both as regards clinical and laboratory diagnosis, and from the point of view of owner-psychology. Nevertheless, its incidence is probably increasing, both absolutely, owing to husbandry tendencies, and relative to the infectious type. Few workers have succeeded in placing their diagnoses on anything approaching satisfactory analytical bases, but where this has been done (by 4 specialist clinicians) the incidence of purely functional problems has varied from 65 to 83 per cent. on a basis of 641 problems. Classification has been attempted both by symptoms and by causes, though the typical outbreak may well provide examples of most symptom-syndromes and result from the synergistic effect of several of the likely causes ; the latter include breed susceptibility, for example the wide variation in feed-conversion ability evidenced by Jerseys as contrasted with Friesians (Lousse & Cordiez, 1954) ; genetic effects, including cystic ovarian disease and possibly gonadal hypoplasia (both of which are linked with good yielding capacity) as well as some forms of early foetal death ; seasonal effects—mainly rainfall—regional effects ; and management, which may be the most important.

The imposition of too short an interval between calving and first service is a common mistake, as evidenced by controlled experimental results on broad bases from America, Britain and Scandinavia; as is also the attempt too rapidly to increase the average yield, from a herd recently established with cheap foundation stock. There is positive evidence that high yield can prejudice fertility (Rottensten, Buch & Carman). Controlled field evidence has been obtained (Dawson, 1954) that outbreaks of foetal resorption can be due to *marginal* progesterone deficiency, and this condition has been clearly reproduced in experimental cattle, also between 2nd and 5th months (Raeside & Turner, 1951). The current rise in incidence in organism-free abortions may reflect in this country the effects of Buch's American observation, that twice as many palpable pregnancies between the 35th and 150th day, were lost in above-average as below-average yielders (progesterone being necessary for mammary development). Other successive degrees in deviation from the functional normal, include early foetal death (under 21 days), imperfect luteinisation, delayed ovulation, anovulatory cycles and cystic ovarian disease ; there is evidence (Dawson, 1957) to suggest that the latter's incidence may have doubled in the course of the last five years.

It is considered that complete abeyance of the ovarian cycle leads frequently if not usually to a sterile "purulent" endometritis, which tends to become secondarily infected with *C. pyogenes* (Dawson, 1951, 1953) and to ascend the tract to produce salpingitis and bursitis (incidence 53 per cent. of barreners, Dawson, 1956). McDonald (1954) demonstrated a link between this sequence and failure of progesterone metabolism, acting through placental retention.

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THE ANIMAL HEALTH TRUST

At the meeting of the Trustees and Council of The Animal Health Trust held on December 6th at Stock, at which the Presidents of the R.C.V.S. and the B.V.A. were present, the President, the Duke of Norfolk, stated that the Trust could now look forward to the future with great hope. In particular, he felt that the meeting would agree that the confidence of the farming world as a whole was being gained. Looking back to the precarious financial position the Trust was in a few years previously, it would be realised that a debt of gratitude was owed to Lord Rank for all he had done, and was doing, on its behalf. The President also congratulated the Scientific Director on the report of the work of the Trust over the last 2 years.

Lord Rank, in giving his report as Chairman of the Executive Committee, stated that a film première was being organised on behalf of the Trust on February 6th, 1958, and he hoped that at the end of the year the overdraft, which still remained large, would be reduced to very much smaller proportions. He emphasised that more money was still required for the Trust's educational work, and also particularly for the canine side of its activities.

The following members of Council, who retired by rotation, were re-elected:—

Sir Weldon Dalrymple-Champneys, Mr. F. S. Gentle, Lord Stamp, Sir James Turner, and Air-Commodore J. A. Cecil Wright (Chairman of the Kennel Club). Lord Northesk was elected a new member on the proposal of Lord Rank, seconded by the Duke of Norfolk.

The Control of Bovine Vibriosis*

BY
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SUMMARY.—Vibrio fetus *infection is a disease causing temporary infertility in cows and is spread by infected bulls either at service or by artificial insemination. Diagnosis in bulls is most satisfactorily made by recovering* Vibrio fetus *from the vaginal mucus of known vibrio-free heifers after insemination. In a herd, diagnosis is made on the herd history and the results of the mucus agglutination test. Wherever possible the organism should also be isolated from aborted foetuses or vaginal mucus samples.*

The disease in the bull can be successfully treated using antibiotics applied locally to the sheath. Since the disease tends to be self-limiting in the female and is transmitted by infected bulls at coitus or by artificial insemination, the most effective method of control is to use artificial insemination on all cows using known non-infected bulls or infected bulls that have been treated and subsequently proved to be free of infection. The addition of antibiotics to infected semen and the dilution effect do not eliminate completely the possible risk of transmitting the disease.

THE control of an infectious disease is based on a study of the aetiological agent, the mode of infection and method of spread both within a community and from one community to another, accurate methods of diagnosing the disease and means of treating it once outbreaks have occurred or preventing outbreaks by immunisation or other means.

M'Fadyean and Stockman (1913) and Smith (1919) isolated *Vibrio fetus* from aborted foetuses but it was not until much later that it was shown that the main feature of the disease was a temporary infertility necessitating numerous services or inseminations for conception and that the disease was transmitted either venereally by the bull at coitus or by artificial insemination.

Aetiology

Although *V. fetus* has been known for over 40 years, it is only within the last few years that any real attention has been paid to a study of the organism itself. Impetus has been given to these studies since it has been found that vibrios other than *V. fetus* can be found in the bovine genitalia. In this connexion, particular mention must be made of the work of Thouvenot and Florent (1954), Bryner and Frank (1955), Price, Poelma and Faber (1955), and Kuzdas and Morse (1956). Bovine vibrios require a reduced oxygen tension for growth which is slow and never profuse. They are actively motile by means of single, polar flagella. Morphologically, *V. fetus* presents the typically spirillar forms but it has been found by the authors that the catalase negative strains consist almost entirely of single, comma-shaped forms. They are biochemically inert, but *V. fetus* is catalase positive and H_2S negative whereas the non-pathogenic vibrios are catalase negative and produce abundant H_2S. There is no serological relationship between the two catalase groups. Therefore, the identification of *V. fetus* and its differentiation from other vibrios is now on a sounder basis. Although *V. fetus* as a species is pathogenic, it is not known whether avirulent strains occur in nature. The main differences between *V. fetus* and the non-pathogenic vibrios can be summarised as follows:—

TABLE I
SUMMARY OF MAIN DIFFERENCES BETWEEN *Vibrio fetus* AND THE NON-PATHOGENIC VIBRIOS

	Catalase production	H_2S production	Reduction of nitrates to nitrites	Morphology
V. fetus	+	—	+	Short and long spirals
Non-pathogenic vibrios	—	+	+	Short comma forms

Mode of Infection and Spread

Terpstra and Eisma (1951) and Lawson and MacKinnon (1952) in a series of classical experiments, showed that the female became infected following service by an infected bull, or by being inseminated with semen of an infected bull. Bulls became infected by serving infected cows. Although this is the main method of infection and spread, there is no doubt that bull to bull spread of infection can and does occur, *e.g.* in a bull stud (Adler, 1956). The consensus of opinion seems to be that infection by contact exposure does not occur in the female although Plastridge and his co-workers (1955) reported that this does. Bulls remain infected for many years and the seat of infection appears to be the mucosa of the penis and prepuce. Rasbech (1952), however, reported the urinary organs of one bull to be infective but this report has not been confirmed. Likewise, in the female, the disease is confined to the genital organs but, unlike the bull, many cows lose the infection some months after the onset of the disease although some remain carriers even after a normal gestation.

There is no doubt that the disease can be transmitted by the use of artificial insemination using an infected bull, but the extent to which it is spread by this means is not known.

There is no intermediate host acting as reservoir of infection or concerned with transmission.

* Paper presented by the Senior Author (W.J.B.M.) to the Society for the Study of Animal Breeding, London. Oct. 1956.

Diagnosis

In the Bull

The disease in the bull presents no symptoms nor lesions either microscopic or macroscopic. Since, however, the infected bull is the natural reservoir of the disease and is responsible for spreading it to the female either at service or by artificial insemination, a reliable and quick means of diagnosis is essential.

Diagnosis by direct culture of semen and preputial samples has not proved reliable, largely because culture plates become overgrown with fast-growing contaminants.

American workers claim some success in isolating *V. fetus* from semen samples using a semi-solid medium (Frank and Bryner, 1953; Hughes, 1956). Similarly Florent and Vandeplassche (1956), using a brilliant green selective medium, obtained good results in isolating *V. fetus* from semen samples. Semen samples and preputial swabs from 26 bulls at this Centre were repeatedly cultured on Thio blood agar plates in 10 per cent. CO_2. Vibrios were recovered from the preputial swabs of 20 of the bulls and the semen of 12. Twenty of these strains were obtained in pure culture and examined biochemically. In all cases, they were catalase negative and H_2S positive.

It was later shown that 3 of these bulls were carriers of *V. fetus* but the organism was not recovered from either the semen or preputial samples.

The use of either pregnant or oestrous guinea-pigs proved unsuccessful for diagnosis although South African workers have reported excellent results (Van Rensburg, 1956).

Adler in a series of papers (1952, 1954b) reported the successful diagnosis of Vibriosis in bulls by inseminating one heifer per bull and culturing biopsy material from the uterus and cervix. This technique, with some modifications, has formed the basis of a method of diagnosis which has been used at Reading. Briefly the method was as follows: heifers were bought and the vaginal mucus cultured and subjected to the mucus agglutination test on at least 3 occasions before insemination, only heifers which were culturally and serologically negative for *V. fetus* being used. Corpora lutea were expressed from suitable heifers, and any showing subsequent signs of oestrus were inseminated with 2 ml. semen into the uterus, using one heifer per bull. Commencing at a week after insemination, uterine biopsy and anterior vaginal mucus samples were collected and cultured twice weekly until 6 examinations had been made. Negative heifers were subsequently reinseminated with the semen of another bull.

Any vibrios were subcultured and pure cultures examined biochemically and serologically. Mucus agglutination tests were carried out regularly every 2 weeks. The results of these examinations are shown in Table II.

The 13 bulls listed in the Table were diagnosed as carriers of *V. fetus*. Fifty-two per cent. of the biopsy samples and 90 per cent. of the samples of vaginal mucus were culturally positive for *V. fetus*. In all cases the organisms were catalase positive and H_2S negative, except for two strains where there was a slight browning of the lead acetate paper. Formalised suspensions of all these strains were agglutinated by stock sera of *V. fetus*.

TABLE II
RESULTS OF CULTURAL AND BIOCHEMICAL EXAMINATIONS

Bull	Heifer No.	Cultural results +i		Biochemical properties		
		Uterine biopsy	Vaginal mucus	Catalase	H_2S Prod	Reduction of nitrates to nitrites
G17	76	5/6	6/6	+	−	+
F2	77	5/6	5/6	+	−	+
F2	65	5/6	6/6	+	−	+
F10	78	4/4	6/6	+	−	+
S1	48	2/6	6/6	+	−	+
F1	60	5/6	6/6	+	−	+
A1	70	4/6	6/6	+	−	+
H1	87	2/6	6/6	+	−	+
A2	56	2/6	5/6	+	−	+
S2	75	2/6	4/6	+	−	+
F4	59	1/6	5/6	+	−	+
F5	55	0/6	3/6	+	(−)+ 2	+
S3	94	3/6	5/6	+	(−)+ 2	+
H2	98	3/6	6/6	+	−	+
		43/82 = 52%		75/84 = 90%		

+ i Denominator = No. of samples cultured.
Numerator = No. of samples positive for *V. fetus*.
+ 2 (−) = Slight browning of lead acetate paper.

Twenty-nine other bulls were similarly examined. From the majority of the heifers inseminated with the semen of these bulls, no vibrios were recovered but catalase negative and H_2S positive vibrios were isolated from some. These catalase negative vibrios were never recovered regularly from any particular animal.

From 4 heifers inseminated with the semen of 4 different bulls, *V. fetus* was recovered on one occasion only from each heifer. All subsequent examinations were completely negative in spite of repeated examinations being made. Whether these were avirulent strains of *V. fetus* that were quickly killed off in the vagina or the result of accidental contamination of the particular samples is not certain.

It is not known whether an infected bull is infectious at all times or whether heifers resistant to infection exist. Therefore, bulls which were negative at the first examination were re-examined twice at 6-monthly intervals. In no case was *V. fetus* recovered from any of the inseminated heifers.

Diagnosis in Cows and Heifers

In recently infected cows the main symptoms are return to oestrus with animals requiring numerous services per conception. The length of the oestrous cycle is often irregular, varying from 28 to 30 days, and there is frequently an increased flow of mucus. The main lesions are a cervicitis and endometritis. However, none of the symptoms or lesions is characteristic of the disease and a diagnosis should be based on laboratory findings.

The mucus agglutination test has proved much more reliable than the serum agglutination test for

diagnosis. It is specific for *V. fetus* infection and has proved to be a valuable tool in diagnosis. Like all other serological tests, the information it gives is limited—a positive result means that the animal has responded to an antigenic stimulus—in this case, *V. fetus*. It gives no information as to when the animal first became infected, whether the animal is in the early stage of the disease and likely to require many services or inseminations for conception, or whether the animal has acquired a good resistance to the disease and conceives normally. Therefore, a positive mucus agglutination test and normal conceptions are not incompatible in a herd. Obviously the result of the test must be interpreted in the light of the herd history (Boyd, 1955).

In other infections, a rising or falling blood titre can give valuable information as to the progress of the disease but the very physical nature of vaginal mucus makes any accurate estimations of titre difficult.

Cultural examinations of foetuses and foetal membranes are a reliable and accurate means of diagnosis where the material is available in a fresh state but the abortion rate is never high, varying from 2 to 12 per cent. From the published results in America, catalase negative vibrios have not been isolated from aborted foetuses.

Cultural examination of anterior vaginal mucus with the isolation of *V. fetus* affords proof of infection but it must be remembered that the duration of infection in the female is variable. It has been found by the authors that in most cases, in experimentally infected heifers, the organism can be easily recovered from vaginal mucus from about 7 days until about 4 months after infection, irrespective of the stage of the oestrous cycle. After this period isolation becomes more difficult and, in many cases, it is not possible to recover the organism after about 6 to 7 months. Occasionally the organism has been isolated after a normal gestation (Frank & Bryner, 1953; Lawson & MacKinnon, 1953). This fact is of great importance in control.

In a herd suspected of being infected with *V. fetus*, diagnosis should be made on the basis of the herd history and on the results of the mucus agglutination test, and whenever possible, the organism should also be isolated from the aborted foetuses, or from the vaginal mucus of recently infected animals.

Control Measures

As mentioned earlier, infected females quickly acquire a tolerance to the disease, although the exact duration of this tolerance, in the absence of reinfection, is not known. On the other hand, bulls once infected appear to remain so for life. These two factors, together with an understanding of the mode of infection, form the basis for a control programme.

Once the disease has been diagnosed either in a herd or bull stud, the most effective method of control is to discontinue using the infected bull or bulls and to use artificial insemination from a known non-infected bull on all the animals in the herd. The greatest precautions must be taken to ensure that the non-infected bull or bulls do not become infected—they must not be allowed to serve any animal naturally; another male should be used as a teaser and the general hygiene with regard to the bull pen and the sterilising of the artificial vagina and other instruments must be satisfactory. New bulls must be shown to be free from infection before being used and, in the case of a bull stud, periodic checks should be made to ensure freedom from infection.

Another method is to divide the herd into two groups: Group A consisting of all the virgin heifers and Group B consisting of the rest of the herd. Animals comprising Group A are served naturally with a non-infected bull, and animals of Group B are inseminated artificially. This method is also satisfactory again as long as precautions are taken to ensure that the bull used for serving virgin heifers is not allowed to serve any of the other cows.

Since it would not always be practical to discontinue using infected bulls, consideration must be given to their treatment. It is not proposed to enumerate here the many references in the literature concerning treatment but the results obtained in the treatment of 13 bulls diagnosed as carriers of *V. fetus* will be described.

Two antibiotic suspensions were used. (1) An antibiotic infusion consisting of 2 g. streptomycin and 1 million units penicillin in an oily base the volume being made up to 100 ml. (2) An antibiotic cream also consisting of 2 g. streptomycin and 1 million units penicillin made up to a 100 ml. and dispensed in a collapsible metal tube.*

The bulls were restrained and, after washing the preputial opening with a 1 per cent. cetrimide† solution, the antibiotic suspension was introduced into the posterior third of the sheath by injecting it through a sterile catheter with a syringe. A gauze bandage was tied round the preputial opening and the antibiotic suspension distributed throughout the sheath by massaging for one minute every 10 minutes for an hour. The bandage was then removed and the bulls placed in thoroughly disinfected pens with clean bedding. This treatment was repeated once daily for 3 days.

Seven bulls were treated using the antibiotic infusion and 6 with the cream. A heifer was inseminated with the semen of each bull at 2 weeks, 2 months, and 6 to 7 months after treatment. One bull had to be disposed of 2 months after the treatment, and 2 bulls have been examined at 9 months after treatment.

Cultural examinations were made of vaginal mucus of the heifers after insemination, and any vibrios isolated were examined biochemically. The results are summarised in Table III overleaf.

Apart from one culture of *V. fetus* isolated from one heifer on one occasion only, all the heifers were culturally negative for *V. fetus* after insemination with the semen of the treated bulls and this contrasts strikingly with the picture before treatment. Our experience confirms that of other workers that infected bulls can be successfully treated for Vibriosis. This is of considerable importance since the success-

* The antibiotics were supplied by Glaxo Laboratories, Ltd.
† Cetavlon.

TABLE III
CULTURAL RESULTS FROM HEIFERS INSEMINATED WITH THE SEMEN OF BULLS PRIOR TO AND FOLLOWING THEIR TREATMENT
PRESENCE OR ABSENCE OF INFECTION IN INSEMINATED HEIFERS

Before Treatment	After Treatment			
Bull	2 weeks	8 weeks	6 to 7 months	9 months
F2 +	−•i	−	−	−†ii
F10 +	−	−	Not done	
F1 +	−	−	−†ii	
A2 +	−	−	−†ii	
S2 +	−	−	−	
F4 +	−	−	−†ii	
S3 +	−	−	−	
G17 +	−	−	−	−
S1 +	−	−	−	
A1 +	−	−	−	
H1 +	−†ii	−	−†ii	
F5 +	−	−	−	
H2 +	−	−	−	

Of the 13 bulls tested in Table III 10 of them are still alive and were found to be free of *Vibrio fetus* infection 2 years after treatment.

• i *V. fetus* recovered on one occasion only.
† ii Catalase negative vibrios recovered.
Bulls F2 to FH treated with antibiotic infusion.
Bulls G17 to H2 treated with antibiotic cream.

ful treatment on one bull can prevent possible spread of infection to numerous females.

The question of the treatment of infected cows and heifers, especially on a herd basis, is not so important since many females appear to develop a resistance to the disease and eventually conceive. Lawson (1954) reported the successful treatment of infected heifers by using chlortetracycline* as a local application to the uterus and vagina. Results using streptomycin or penicillin were not so satisfactory. Adler (1954), however, reported best results using streptomycin. If treatment of the cows is contemplated, it should be instituted in the early stages of the infection before immunity has developed.

The addition of antibiotics to semen diluents is widely employed in artificial insemination. The conception rates of some, but not all, bulls are significantly increased by the addition of antibiotics, but certainly not all such bulls are infected with *V. fetus*. Frank and Bryner (1953), Orthy and Gilman (1954), and McEntee, Hughes and Gilman (1955) claim that, by the addition of 500 μg. per ml. streptomycin, 500 μg per ml. penicillin + 3 mg. per ml. sulphanilamide to infected semen diluted at least 1/25 and held for at least 6 hours at 5° C. before use, such semen will not infect inseminated heifers. Lawson (1954), however, was still able to infect 5 out of 23 heifers after insemination with semen containing penicillin, streptomycin, and sulphanilamide in the above concentrations held at room temperature for 2 hours before being stored at 4° C.

The authors' experience is in accordance with that of Lawson. Semen from a known infected bull was treated as follows:—

A. Undiluted semen with no antibiotic.

B. Semen diluted 1/5 in egg-yolk citrate buffer + 500 μg. per ml. streptomycin.

C. Semen diluted 1/50 in egg-yolk citrate buffer + 500 μg. per ml. streptomycin.

* Aureomycin.

After dilution, the semen samples were left at 5° C. for 2 hours before being used for insemination. The results were as follows:—

Heifer No.	Inseminated with:	No. of *V. fetus* cultures obtained from		No. of positive results to the mucus agglutination test
		Uterine biopsy samples	Samples of oestrous mucus	
21	Undiluted Semen	1/6	4/9	3/11
19	Semen diluted 1/5 + 500 μg per ml. streptomycin	0/5	0/3	0/11
20		3/6	2/4	6/10
41				
32	Semen diluted 1/50 + 500 μg per ml. streptomycin	0/5	0/7	0/9
33		3/6	3/5	3/9

Under the conditions of this experiment neither the dilution rate nor the addition of 500 μg. per ml. streptomycin prevented the heifers from becoming infected.

Little can be said on control by immunisation. Osborne (1952) using an avianised vaccine reported increased conception rates in the vaccinated over the control animals. Easterbrooks, Plastridge, Williams and Kiggins (1955) reported a killed *V. fetus* vaccine to be of no value, but mentioned that a live vaccine was giving encouraging results. It is debatable, however, whether immunisation can contribute materially to the control of a disease of this sort bearing in mind the mode of infection and spread. The only solution is complete eradication.

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The Effect on Embryonic and Chick Mortality of Yolk Contamination with Bacteria from the Hen*

BY

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SUMMARY.—The death of embryos and chicks can result from an infection of the yolk by a number of bacteria of types frequently present in the alimentary tract, and on the skin of the hen. Two outbreaks, however, have been investigated in which Staph. aureus *of human origin has been the cause of yolk infection.*

(2) *Yolk infection in chicks is usually initiated by bacteria, relatively non-pathogenic in sites other than the yolk, which possess enzymes capable of degrading the yolk protein complexes, and occasionally by toxigenic bacteria capable of stimulating the production of an inflammatory exudate from the yolk sac lining. Other contaminants present subsequently contribute to the infection.*

(3) *Yolk infection in embryos is usually attributable to motile types of bacteria, mortality being highest during the first 2 days of incubation.*

(4) *In infected chicks of relatively high resistance, death is usually accompanied by the production of macroscopic lesions, the yolk being coagulated and the yolk sac haemorrhaged to varying degrees. In susceptible infected chicks and embryos, death occurs before the production of macroscopic changes in the yolk. The existence of an infection in these cases can be established by histological examination of the yolk and yolk sac.*

(5) *Yolk contaminants, in the embryo, are principally those which have originated from the alimentary tract, or the skin of the hen, and have migrated from the shell where they were originally deposited.*

(6) *The source of yolk contaminants in the chick, apart from bacteria pre-existing in the yolk before hatching, is principally the alimentary tract of the chick, from which bacteria migrate during the first few days after hatching.*

(7) *Factors contributing to fatal yolk infections are a low standard of hygiene on the supply farm and hatchery, which tends to increase the possibility of contamination with bacteria capable of initiating infection, and sub-optimal conditions of incubation and storage and brooding of chicks, which delays yolk absorption and lowers resistance to infection.*

The Control of Bovine Vibriosis.—*Concluded.*

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BACTERIA, of the types normally found in the alimentary tract and on the skin and feathers of the hen, are present in large numbers on and in the shells of eggs (Haines, 1938), and thus come into close contact with embryos and chicks in the incubator. It has been found that this frequently results in these organisms gaining access to the yolk.

"Mushy Chick" Disease

Lesions

The possibility of such yolk contamination developing into a fatal infection, first became apparent during investigations on the aetiology of the condition in chicks known as "Mushy Chick" disease, or yolk sac infection. This condition, which can occur in chicks at any period extending up to 10 days from the time of hatching, is characterised by an inflammation of the yolk sac and is usually accompanied by a denaturation of the yolk which is transformed from its normal greenish yellow translucent emulsion, either to a brownish turbid fluid, or to a granular or solid refractive yellow mass, the type of denaturation being to some extent dependent on the nature of the infecting organisms.

Economic importance

The extent to which chick mortality occurred from yolk sac infection during the main hatching season

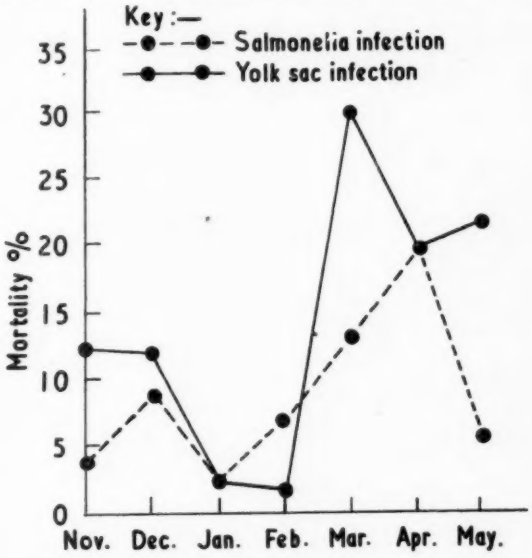

FIG. 1.

* Paper presented to the summer meeting of the British Veterinary Poultry Association, Cambridge, June, 1957. Details of experimental work will be published elsewhere.

of 1955, is shown in Fig. 1 which was compiled on a monthly basis from *post-mortem* records, and in which the incidence of mortality from yolk sac infection is compared with that from Salmonella infections.

In the peak month 129 chicks, representing 32 outbreaks, were received showing the clinical symptoms of yolk sac infection. The majority of these chicks were aged 1 to 4 days, having died before transit from the hatchery, or within a few days of being placed in the brooder. In no instance was a subsequent peak of mortality reported in any of these outbreaks, thus implying that no infectious agent, transmissible from chick to chick, was involved. The flock mortality in these outbreaks was usually in the region of 5 per cent. and seldom exceeded 10 per cent.

Cases of true omphalitis or "Navel-Ill" which are characterised by a necrosis and oedema of the tissues in the proximity of the navel, and are associated with imperfectly healed navel scars and adhesions with underlying tissues, were rarely encountered in the chicks examined, in which the infection was confined to the yolk sac.

Isolation of bacteria from lesions

Investigations on the aetiology of yolk sac infection were directed initially at isolating and identifying the various bacteria present in the yolk of infected chicks. A study of the bacterial flora of the yolk was made not only on chicks which had died showing obvious abnormalities of the yolk and yolk sac, but also on dead chicks in which the yolk appeared macroscopically normal. The results of this investigation are shown in Table I.

TABLE I

THE FREQUENCY WITH WHICH VARIOUS GROUPS OF BACTERIA CONSTITUTE A MAJOR PART OF THE YOLK FLORA IN DEAD CHICKS

Bacterial groups isolated from yolk	Per cent. incidence per chick	
	Denatured yolk	Normal yolk
Escherichia	82	52
Aerobacter	6	2
Proteus	16	9
Salmonella	8	3
Bacillus	18	0
Micrococcus	25	11
Streptococcus	53	22

It was considered initially that the absence of macroscopic abnormalities in yolk sacs and yolk precluded the possibility of a yolk sac infection, so that the bacterial content of such yolks in dead chicks would indicate the nature and extent of *post-mortem* contamination. Subsequent observations, however, on embryonic infections of the yolk, indicated that under certain circumstances, such as where the infecting organisms were devoid of enzyme systems capable of disrupting the yolk, or where the chicks died in the early stages of the infection, it was possible for death to occur from a yolk sac infection in the absence of premonitory symptoms, or of macroscopic abnormalities of the yolk or yolk sac. Although giving some indication of the possible nature and extent of *post-mortem* contamination, the bacterial flora of apparently normal yolks from dead chicks cannot therefore be considered to consist exclusively of contaminants of *post-mortem* origin.

It was found that the abnormal yolks were characterised by having a more diverse flora per chick than those of the other type. In approximately 95 per cent. of 240 abnormal yolks examined, bacteria of more than one species were present in large numbers. Another characteristic of the abnormal yolks was the relatively high incidence in them of Proteus type organisms and aerobic sporing bacilli. In the majority of the 171 apparently normal yolks examined only a single species of bacteria was isolated. The degree of contamination was also less than in abnormal yolks, and from 16 apparently normal yolks examined, no bacteria were isolated by direct plating. Anaerobic culture of 48 decomposed yolks resulted in the isolation from 15, of *Clostridium histolyticum, sporogenes, tertium* and *welchii* type A. Pseudomonads, having an optimal growth temperature of 25° to 30°, were frequently isolated from abnormal yolks but were never found to be the predominating organisms present. It is considered possible, that in some cases pseudomonads may have predominated in the early stages of infection but were subsequently supplanted by coliforms having an optimal growth temperature more closely related to that of the chick.

The results showed that no specific bacterium was invariably associated with the production of yolk and yolk sac abnormalities, and this finding applied, in the majority of cases, to individual outbreaks. An exception to these findings occurred in 2 outbreaks of yolk sac infection, in which *Staph. aureus* predominated in the yolks of all the chicks examined.

The incidence of bacteria of recognised pathogenicity, which are absent from the alimentary tract and skin of the healthy fowl, was found to be low. Using non-selective media, the incidence of Salmonellae in the 240 abnormal yolks examined was 8 per cent. and of the micrococci isolated only 2 per cent. were found to be *Staph. aureus*. In 191 similar yolks, cultured on selective media, the incidence of these organisms was 26 per cent. and 11 per cent. respectively. It is considered that the increase in the incidence of these organisms resulting from the use of selective media, implies that in most cases they were present in the yolk in relatively low numbers and could possibly have originated from lesions elsewhere in the chick. In the case of chicks with Salmonella-infected yolk, lesions were often found on the heart and in the lung tissue.

Specific characteristics of yolk-infecting bacteria

A further examination was made of the various organisms isolated from abnormal yolks, for characteristics which would contribute to yolk decomposition and the death of the infected chick, such as the possession of enzyme systems capable of using the intact yolk as a substrate, or the liberation of toxins capable of producing an inflammatory action on the yolk sac wall and a generalised toxaemia.

The degree to which bacteria, representative of the various groups isolated, were able to decompose susceptible yolk samples is shown in Plate 3. Organisms

E. G. HARRY.—THE EFFECT ON EMBRYONIC AND CHICK MORTALITY OF YOLK CONTAMINATION WITH BACTERIA FROM THE HEN.

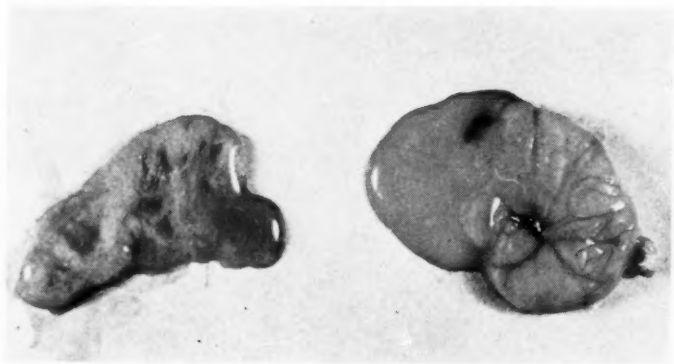

PLATE 1.—A normal and infected yolk sac torn open to expose the yolk. The vitelline veins in the infected yolk sac are dilated and the yolk has the characteristic curdled appearance which is produced by extensive bacterial proliferation.

PLATE 2.—The yolk sacs of chicks which have died from an experimental infection with pure cultures of toxigenic bacteria exposed to show the dilatation produced in the vitelline veins and the increase in size of the yolk sac, resulting from the inflammation of the cells constituting the yolk sac wall, by bacterial toxins. Top: *Staph. aureus, Salm. pullorum.* Bottom: *B. cereus,* Normal.

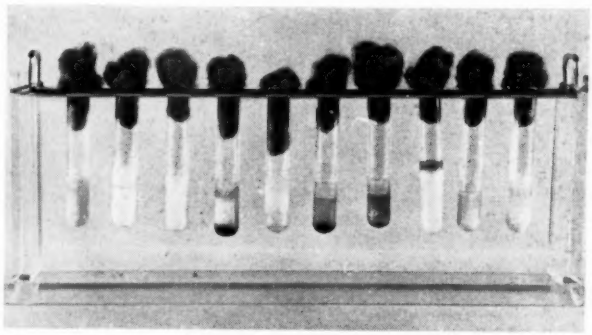

PLATE 3.—The changes produced in egg yolk as a result of the growth of various yolk sac contaminants after 2 days' incubation at 37° C. From the left: Uninoculated control, *B. cereus, Staph. aureus, Cl. sporogenes, Cl. histolyticum, Cl. tertium, Pr. vulgaris, Ps. aeruginosa, E. coli, Str. zymogenes.*

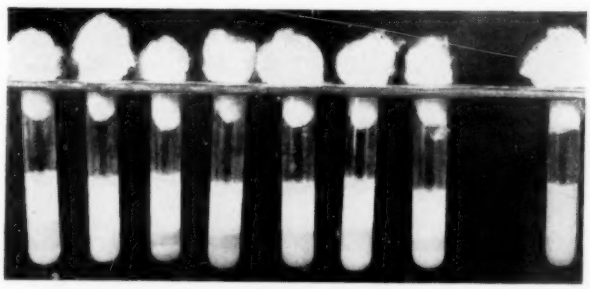

PLATE 5.—The changes produced in samples of yolk from different eggs diluted with an equal volume of water, after inoculating with equal numbers of *B. cereus* and incubating at 37° C. for 2 days. (End tube, at right, is an uninoculated control).

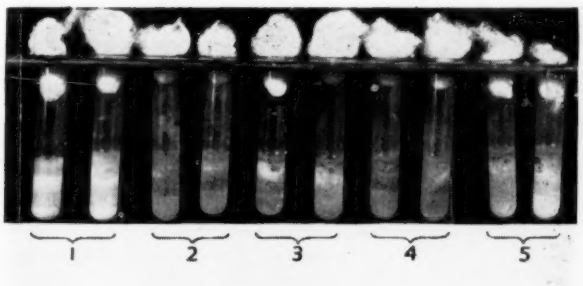

PLATE 4.—The changes produced in duplicate samples of yolk from 5 eggs, after inoculating with equal numbers of *B. cereus* and incubating at 37° C. for 2 days.

of the coli-aerogenes group, Salmonellae, enterococci, and coagulase negative staphylococci, were only found to multiply to a limited extent and produce only slight changes in yolk, which is separated by them into a clear supernatant portion and a lower opaque portion. With certain coliforms, a small amount of gas is produced from carbohydrate fermentation. The greatest amount of change is shown to occur with *B. cereus* and *Cl. sporogenes*, which are known to possess a lecithinase (McGaughey & Chu, 1948; McClung, 1946), *Staph. aureus*, which possesses a lipase enzyme system (Gillespie & Adler, 1952), and by *Pr. vulgaris* and *Ps. aeruginosa* which possess protease enzymes (Haines, 1939). Total cell counts on yolk samples inoculated with these organisms, showed that the extent of bacterial multiplication was directly related to the degree of decomposition of the yolk. Bacteria with enzyme systems capable of disrupting and degrading the yolk lipoproteins are therefore able to multiply extensively when present in the yolk of the chick and also allow the proliferation of other species of bacteria present. It is possible for such bacterial proliferation to produce pathogenic reactions in the chick, even when the virulence of the toxins, capable of being produced by the bacteria concerned, is relatively low. Bacteria non-pathogenic in sites where proliferation is restricted, may become pathogenic when present in the yolk.

These *in vitro* tests on yolk growth, were made in both egg yolk and embryonic yolk, and it was found that undiluted yolk was a relatively unfavourable growth medium, even for those organisms which possessed lecithinase or lipase enzyme systems, yolks from certain eggs being found more growth inhibiting than others. This variation of individual yolk samples, in ability to support bacterial growth, is shown in Plate 4, which demonstrates the differences in the amount of growth and coagulation produced in yolks, taken from different eggs, when inoculated with identical numbers of *B. cereus*.

This variation in growth promoting ability of different yolks appears to be primarily associated with the amount of free water available, for when samples of yolk from different eggs were diluted 50 per cent. with water and inoculated with *B. cereus*, as before, an identical and increased amount of coagulation occurred in each yolk sample, as shown in Plate 5.

These investigations of the growth characteristics of bacteria in yolk, established a relationship between the clinical type of yolk sac infection and those bacteria which are able to disrupt and degrade the yolk lipoproteins, a higher incidence of these bacteria being found to occur in abnormal yolks. The tests also showed, that decomposition of yolk depends, not only on the presence of certain types of bacteria, but also on the physico-chemical composition of yolk, in particular on its degree of hydration. For this reason, factors such as incubational environment which influence the degree of hydration of embryonic yolk (Byerly, 1932 & Romanoff, 1930), will also tend to influence the progression of an infection.

As soon as bacterial growth is established in the yolk, the degree of hydration will be increased by the inflammatory exudate, which is produced by the cells lining the yolk sac in response to the liberation of the products of metabolism and lysis of the parasitic bacteria. It is considered, that for this reason, once multiplication of toxigenic bacteria is initiated in the yolk, infection then proceeds with great rapidity and any other bacterial types present, which alone would be incapable of establishing growth, can also multiply and probably contribute to the yolk decomposition or toxin production. *In vitro* tests with inoculated yolk actually showed that decomposition of the yolk by *Bac. cereus* proceeded more rapidly when the yolk was simultaneously contaminated with *Str. faecalis*. This probably explains the existence of the more diverse bacterial flora encountered in decomposed yolks. A diverse flora in the yolk is probably for this reason a cause as well as an effect of yolk sac infection in chicks, and represents secondary as well as primary infecting agents.

The toxigenicity of the various species of bacteria isolated from abnormal yolks, was determined by *in vitro* tests on their proteolytic and haemolytic activity, and by *in vivo* tests on their pathogenicity in chicks.

It was found that the majority of the organisms of the coli-aerogenes group isolated were able to clear blood agar plates when a heavy inoculum was used, but none were able to lyse horse or chicken red cells when tested by the tube method, or to liquefy gelatin. No serological examination of these organisms was made. None of the 15 aerobacter group organisms isolated were haemolytic by plate or tube test, or were proteolytic. All of the 20 Proteus organisms isolated, produced 100 per cent. lysis of horse and chicken red cells, and 16 were proteolytic. The streptococci isolated were all of the enterococcus group, and of 16 cultures examined, 7 were proteolytic having the characteristics of *Str. liquefaciens*, and 7 produced β-haemolysis on blood agar plates, having the characteristics of *Str. zymogenes*. The organisms in the Bacillus group isolated, were principally *B. cereus* and *B. mycoides*. Of 25 cultures tested, 9 produced 100 per cent. lysis of horse and chicken red cells, 1 produced 50 per cent. and 4 produced 25 per cent., the remainder being non haemolytic. Marked haemolytic activity was usually found to be associated with yolk coagulating ability. Of 62 cultures of micrococci isolated, only two were found to be coagulase positive. These had the characteristics of *Staph. aureus*, and were able to clear blood agar plates but were unable to lyse horse and chicken red cells when tested by the tube method. They were found to be proteolytic, and coagulated yolk. Both, by their phage type, were considered to be of human origin. Identical strains were subsequently isolated from workers at each of the two hatcheries concerned in these outbreaks. None of the coagulase negative micrococci had any haemolytic activity, although the majority exhibited slight proteolytic activity. With the exception of *Cl. tertium*, all the clostridia isolated were proteolytic, *Cl. welchii* and *sporogenes* being haemolytic in addition.

These tests showed that a high proportion of the

organisms isolated from abnormal yolks possessed biochemical characteristics associated with pathogenicity.

In vivo pathogenicity tests were made by inoculating 0.1 ml. of undiluted, and diluted, 18-hour broth cultures into the yolk of groups of 5 to 10 one-day-old chicks. The examination of control chicks, inoculated similarly with sterile broth, showed that death or delay in yolk absorption in the test chicks was due entirely to the effect of bacterial growth. It was found that all the *Staph. aureus*, *B. cereus* and Pseudomonas cultures were capable of producing death in the majority of the chicks, within 2 days of inoculating doses of less than 300 organisms, whereas *Salm. pullorum* exhibited a similar degree of pathogenicity only when inoculated subcutaneously, or into the yolk, in doses of 16 millions. Conversely, doses as large as 300 million cells of *B. cereus*, inoculated subcutaneously produced no pathological reaction, indicating that the lethal activity of this organism was dependant on its multiplication in the yolk. Subcutaneous injections of *Cl. sporogenes* and *Staph. aureus*, also failed to produce lesions in doses which were fatal when administered into the yolk. The tests confirmed that the pathogenicity of these organisms was associated with their ability to multiply in the yolk. Early death was also produced by certain strains of *Str. liquefaciens*, *Str. zymogenes*, *Aerobacter aerogenes* and haemolytic coliforms.

Delayed death, after 2 to 4 days, occurred in all chicks inoculated with proteus species. It was found that organisms of the coli-aerogenes and enterococcus groups, which had no lethal activity when administered individually, produced early death when inoculated collectively into the yolk, possibly, by a synergistic effect. For this reason, the results of pathogenicity tests on individual organisms may not indicate the full extent of their possible pathogenicity in natural infections, in which other types of bacteria are likely to be present in the yolk. The production of pathogenic reactions by bacterial contaminants in the yolk, when these do not include bacteria possessing enzymes utilising yolk, is dependent on the amount of growth required to produce sufficient toxin to stimulate a response in the tissues of the infected chick. This will be at least when bacteria are present which produce toxins of high virulence, such as the Salmonellae, or when the chick infected is in a devitalised condition. A greater amount of growth will normally be required in the case of less toxic organisms, such as the coliforms, enterococci and Proteus species, certain of which are able to cause enteritis, when they succeed in proliferating in high numbers in the human intestinal tract (Dack, 1944; De *et al.*, 1956, & Van Heynigan, 1955). Observations on artificially infected chicks showed, that where death is produced as a result of bacterial proliferation, in the yolk, the direct cause of death is a toxaemia and not malnutrition due to non-availability of the denatured yolk. This is in agreement with a number of reports on the non-essentiality of yolk for the nutrition of the chick (Reyniers, 1949; Urban, 1955, & Romanoff, 1944).

Predisposing factors

It is considered likely, however, that under average farm conditions, which tend to be suboptimal, malnutrition is an important secondary cause of death in the case of infected chicks, as devitalisation, resulting from infection, renders such chicks unable to compete with healthy chicks for the restricted supplies of food and water available, and chicks which have died from naturally acquired yolk sac infection are frequently found to have empty gizzards. Malnutrition, and other stress factors, would probably also tend to produce death in the early stages of infection of the yolk, or induce the development of the clinical features of yolk sac infection. Tests have shown, however, that these factors are unable to induce an active infection of the yolk in the absence of a pre-existent latent infection. Where deaths have occurred in the early stages of infection of the yolk, macrophages, and other phagocytic cells, can occasionally be found in the yolk, together with bacteria in the cells lining the yolk sac.

Diagnosis

The detection of bacterial contaminants in low numbers in yolk of normal appearance in dead chicks, cannot, in the absence of leucocytic infiltration of the yolk, or bacterial infiltration of the yolk sac wall, be regarded as diagnostic of a yolk infection, as bacterial migration from the alimentary tract to the yolk has been found to occur to a limited extent, in live chicks, during the first 2 to 3 days after hatching, and to a greater extent after death. Storage tests on dead chicks, however, showed that bacteria present as contaminants in the yolk at the time of death, or which succeed in migrating into the yolk after death, fail to multiply sufficiently in the yolk to cause its denaturation in chicks stored at 25° C. for 2 days. Denaturation of the yolk was only found to occur when chicks had been kept for 2 days or more at 37° C. The failure of yolk contaminants to multiply rapidly in dead chicks, maintained at temperatures below 37° C., is due to the relative unsuitability of intact yolk for bacterial nutrition as described previously. Rapid growth becomes possible in chicks stored at 37° C. as a result of the extent to which autolysis occurs in the dead chick at this temperature. Yolk denaturation is therefore a reliable diagnostic feature in temperate climates.

Route of infection

In order to determine the routes whereby bacteria gain access to the yolk, investigations were made of the incidence of bacteria in the yolk of the newly hatched chick, and the relationship between possible yolk contamination and bacterial colonisation of the gut in the developing chick. In view of the relative absence of lesions centred on the navel and the failure of attempts made to contaminate the yolk of chicks by applying bacteria to the navel, it was considered that contamination *via* the navel was not of frequent occurrence.

The incidence and degree of contamination of the yolk of day-old chicks, as shown by the growth of bacteria in broth, and on plate subculture, respectively, is detailed in Table II. This shows that both the incidence, and degree of contamination of the yolk is influenced by the degree of contamination of the eggs before incubation.

The relationship of the bacterial content of the yolk to the age of the chick, was determined in chicks of the same hatch at daily intervals after removal from the incubator. The results, shown in Table III, indicate that an increase occurred in the degree of contamination of the yolk after hatching.

A comparison of the bacterial content of the peritoneal fluid, the yolk, and the gut, in chicks of varying ages, showed that a definite relationship existed between them, identical strains of coliform and enterococci being isolated from each of these 3 sites, suggesting that bacteria migrate from the gut to the body tissues duing the first few days after hatching. Confirmation of this migration, was obtained in tests in which specific strains of coliform bacteria were fed to day-old chicks and the subsequent incidence and degree of contamination of the yolk and peritoneal fluid with these organisms was measured. It was found that the migration of bacteria occurred soon after they were introduced into the gut, and the extent of migration began to decline after 3 days accompanied by a decrease in the degree of contamination of the yolk. Bacteria were rarely isolated from the yolks of 6-day-old chicks in these tests, indicating the inability of the initial contaminants to derive sufficient nutrients from the yolk to support continued growth.

TABLE II

THE RELATIONSHIP OF THE BACTERIAL CONTENT OF THE YOLK OF LIVE CHICKS TO THE DEGREE OF CONTAMINATION OF THE EGGS BEFORE INCUBATION. (HATCHES 1 AND 2 CULTURED IN NUTRIENT BROTH, 3 and 4 CULTURED ON NUTRIENT AGAR PLATES)

		Hatch 1	Hatch 2	Hatch 3	Hatch 4
Clean eggs	Bacteria present	8	19	0	0
	Bacteria absent	17	6	25	25
Dirty eggs	Bacteria present	—	21	3	7
	Bacteria absent	—	4	22	18

TABLE III

THE INCIDENCE OF YOLKS CONTAMINATED WITH BACTERIA, IN CHICKS OF VARYING AGE, PRODUCED FROM NATURALLY CLEAN EGGS
(ALL YOLKS CULTURED ON NUTRIENT AGAR PLATES)

	1-day-old		2-day-old		3-day-old	
Batch No.	No. examined	No. contaminated	No. examined	No. contaminated	No. examined	No. contaminated
1	25	0	—	—	—	—
2	25	0	24	1	50	31
3	25	1	25	23	—	—
4	25	10	25	25	—	—
5	25	9	—	—	25	17
6	28	11	30	17	—	—
7	12	3	—	—	12	8
8	22	8	—	—	22	9
9	19	12	—	—	17	8
10	16	4	—	—	—	—
11	20	1	10	1	25	12
12	16	12	—	—	—	—
13	10	3	—	—	—	—
14	25	1	—	—	25	6
Total	293	75	114	67	151	85
Mean % contaminated		25·60		58·77		56·28

The migration of bacteria from the gut apparently takes the form of a generalised invasion of the body tissues *via* the cells lining the gut. It is possible that this migration is associated with an initial susceptibility of the tissues surrounding the gut to invasion by bacteria, which is followed by their sensitisation and acquirement of antibacterial resistance, after the manner described by Enticknap (1956) in the case of the rabbit. No evidence was obtained which suggested that bacteria enter the yolk from the gut *via* the yolk stalk, which, according to reported investigations (Romanoff, 1944; Kaupp, 1918; Lillie, 1952 & Taylor, 1949), does not provide a means of direct contact between yolk and gut contents.

These results show that when yolk infection occurs before the chicks have been removed from the incubator, the source of the infecting bacteria is probably the embryonic yolk. When infection of the yolk occurs 1 or 2 days after removal from the incubator, the infecting organisms can also be those which have migrated from the gut. The factors actually deciding whether such contamination will lead to an infection, are, the extent to which the yolk is contaminated, the virulence or yolk decomposing activity of the bacteria present, the antibacterial resistance of the yolk, and also, the antibacterial resistance of the chick.

Embryonic Mortality

Relationship of preincubation contamination to embryonic mortality

The potential pathogenicity shown to be possessed by certain types of bacteria found in the alimentary tract and on the skin of the hen, when present in the yolk of chicks, suggested that these organisms might have a similar pathogenic potential when present in embryonic yolk. Investigations were therefore made on the effect, on embryonic mortality, of contaminating eggs before incubation, with bacteria obtained from hen faeces, comparison being made with untreated eggs and clean washed eggs from the same source.

The results shown in Table IV, indicate that embryonic mortality can be increased threefold by contaminating eggs with bacteria of faecal origin

TABLE IV

THE EFFECT ON HATCHABILITY OF FERTILE EGGS, OF PREINCUBATION INFECTION, AS DETERMINED ON GROUPS OF EGGS EITHER UNTREATED, OR WASHED IN CLEAN OR FAECALLY CONTAMINATED CHILLED WATER

	Control		Clean washed		Dirty washed	
Test No.	No. set	% hatchability	No. set	% hatchability	No. set	% hatchability
1	54	87	54	76	44	75
2	94	89	87	83	91	66
3	51	83	56	84	49	76
4	51	86	46	91	40	75
5	58	88	58	90	58	78
6	92	91			95	80
All tests	400	87	301	85	377	75

before incubation. Washing eggs in chilled water assists bacteria on the shell to penetrate into the egg contents, but except in the case of the eggs used in one of the tests, the resultant contamination, with the clean eggs used, was insufficient to have an effect on the extent of embryonic mortality. This result may however have been influenced by the incubation conditions used in these tests, which were known to be optimal.

The degree and type of bacterial contamination of the dead embryos in each group was determined in the albumen, allantoic fluid, and yolk. It was found that the higher embryonic mortality in the dirty washed eggs was associated with a higher incidence and degree of contamination of the yolk compared with the eggs of the other groups. All the yolks of dead embryos in the dirty washed groups were heavily contaminated,: whereas 35 per cent. of the yolks of dead embryos in the control groups were sterile by plate culture, and of the remainder, the degree of contamination was slight. The bacteria isolated from the yolks were mainly motile organisms of the *Coli-aerogenes* group. In decomposed eggs Proteus species also were isolated. The age distribution of the dead embryos in these tests is shown in Table V, and indicates that the mortality at the termination of the hatching period is higher in the control and clean washed eggs than in the dirty washed eggs. The embryos dying at the termination of incubation in the eggs of the first two groups had a low incidence of bacteria in the yolk, and their death was presumably due to their lack of vitality, which rendered them incapable of overcoming the stresses associated with emergence from the shell. The fact that a significantly lower percentage of embryos from dirty washed eggs died at this period, suggests that the embryos of low vitality in these eggs died at an earlier age from a bacterial infection, as such embryos would be expected to have a relatively low resistance to bacterial proliferation and its toxic effect. No significant macroscopic difference could be detected between dead embryos of the same age from clean and dirty washed eggs, with the exception of the early dead embryos, which in the dirty washed eggs were frequently decomposed. In addition, no relationship was found to exist between bacterial infection and the occurrence of physical abnormalities in the embryos, such as malpositions and malformations. It is therefore considered that, as in the case of certain chicks, where there is an inherited or induced susceptibility to the toxic effect of infection, death may occur from an infection in which bacterial proliferation has been insufficient to produce macroscopic changes in the yolk. The higher susceptibility of embryos, as compared with chicks, to the toxic effect of infections of the yolk, may be associated with the inability of embryos to manufacture antibodies.

Pathogenicity tests

Tests made to determine the pathogenicity of various species of bacteria to the embryo, have shown that the majority of the bacteria which can be isolated from the faeces or the skin of the hen, are lethal to the embryo when present in the yolk in small numbers (20 to 30 cells per egg). The ability of bacteria, which succeed in migrating into the albumen from the shell, to also penetrate into the yolk, depends on their motility, and ability to resist the antibacterial activity of the albumen. *Staph. aureus* and *B. cereus* for example, which are highly pathogenic in the yolk, have been found to be non-pathogenic when introduced into the albumen. *Salm. pullorum* also, has only a slight degree of pathogenicity when present in the albumen, in contrast to the motile *Salm. thompson*. These tests confirmed the previous observation, that embryonic mortality is associated with the presence of bacteria in the yolk as distinct from the albumen or allantoic fluid, in which bacteria may be present in living embryos, or in dead embryos, which have become contaminated by *post-mortem* migration of bacteria from the shell. In the case of extragenital infection of the egg contents, the potential pathogenicity to the embryo of the various bacteria found on the shell depends principally on motility rather than ability to denature yolk.

Source of egg contaminants

The incidence of bacteria in the yolk of newly laid eggs, has been found to be low. Of 400 eggs from various sources examined, only 2.5 per cent. were found to contain bacteria in the yolk. The incidence of bacteria in ova, however, was found to be much higher. In the absence of further contamination by bacteria penetrating the shell, contaminated yolk in unincubated eggs, tends to regain its sterility owing to the unfavourable nature of yolk for bacterial growth and survival, as mentioned previously.

TABLE V

THE EFFECT OF PRE-INCUBATION INFECTION ON EMBRYONIC MORTALITY AGE DISTRIBUTION. (THE DATA SHOWN ARE DERIVED FROM THE TESTS REFERRED TO IN TABLE IV)

Group	Total number of fertile eggs	Total embryonic mortality	Mortality age distribution, in days													
			1—3		4—6		7—9		10—12		13—15		16—18		19—21	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control	400	47	13	28	0	0	4	9	2	4	0	0	3	6	25	53
Clean washed	301	45	16	36	2	4	4	9	1	2	2	4	4	9	16	36
Dirty washed	377	96	46	48	4	4	10	10	1	1	11	12	8	8	16	17

This is probably the reason why the incidence of bacteria in the yolk of newly laid eggs was found to be less than in ova, congenitally acquired bacteria having succumbed to the bacteriostatic activity of the yolk by the time the eggs were laid.

The most important cause of embryonic yolk contamination appears to be due to bacterial penetration of the shell. Apart from pre-incubation contamination by this route, contamination has also been found to occur on the 18th day of incubation when the eggs are candled and retrayed. In two outbreaks of yolk infection with *Staph. aureus*, which involved both embryos and chicks, the causative organism was traced to the persons involved in handling the eggs at the 18th day, who were found to be either nasal or skin carriers of this organism. Conditions are particularly favourable for bacterial penetration of the shell at this period, as the eggs are cooling at the time of being handled. Another period during which shell penetration by bacteria occurs, is during hatching, when the shells of late hatching embryos are soiled, on the hatching trays, with the blood and allantoic fluid of newly hatched chicks. It has been found, that the mortality rate in embryos soiled in this manner, can be as much as 4 times that of unsoiled embryos, the mortality rate being dependent on the degree of soiling. Tests showed that the death of these embryos is the result of multiplication of contaminating bacteria in the yolk and is not the result of alterations in the texture of the shell as a result of soiling. The high susceptibility shown by embryos of this particular age to infection of the yolk, may be associated with the physiological stresses imposed on the embryo shortly before hatching.

Diagnosis

Owing to the usual absence of macroscopic yolk abnormalities in the embryos which have died as a result of bacterial infection of the yolk, confirmation that death has resulted from a yolk infection usually necessitates an estimation being made of the numbers of bacteria present in the yolk. This is most conveniently carried out by microscopic examination of stained smears of yolk suspensions. In some cases mononuclear and polymorphonuclear leucocytes can also be detected in association with bacteria.

Acknowledgments.—I wish to thank Dr. R. F. Gordon for helpful advice during the course of these investigations, Dr. R. E. O. Williams of the Staphylococcal Reference Laboratory, Colindale for phage typing staphyloccus cultures, Miss J. Wells and Miss S. Day for valuable technical assistance and Mr. C. G. Stacey for assistance in the preparation of the illustrations.

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AREA ERADICATION PLAN FOR TUBERCULOSIS ATTESTED HERDS SCHEME, 1950

Statement showing the position as at October 1st, 1957

	Total cattle on June 4th, 1957 (provisional figures) ('000)	Number of registered attested herds	Total attested cattle ('000)	Percentage of total cattle
GREAT BRITAIN	10,047	180,871	7,353	73
England & Wales	8,252	139,182	5,736	70
England	7,176	102,969	4,751	66
Wales	1,076	36,213	985	92
Scotland	1,795	41,689	1,617	90

1.—COUNTIES WITHIN ATTESTED AREAS—IN WHICH IT IS ASSUMED ALL THE CATTLE ARE ATTESTED

	Total cattle on June 4th 1957 (provisional figure) ('000)		Total cattle on June 4th, 1957 (provisional figures) ('000)
ENGLAND		SCOTLAND	
Berkshire	90	Argyll	71
Cumberland	261	Ayr	168
Isles of Scilly	1	Bute	13
Southampton	161	Caithness	30
Isle of Wight	24	Clackmannan	6
Surrey	61	Dumfries	120
Sussex West	80	Dunbarton	19
Westmorland	108	Fife	77
		Inverness	54
		Kinross	12
		Kircudbright	87
		Lanark	103
		Midlothian	27
WALES		Nairn	11
		Orkney	53
Anglesey	62	Peebles	14
Brecon	54	Renfrew	34
Caernarvon	65	Ross-shire	45
Cardigan	92	Selkirk	8
Carmarthen	157	Stirling	48
Merioneth	40	Sutherland	10
Montgomery	108	West Lothian	18
Pembroke	124	Wigtown	85
Radnor	45	Zetland	7

2.—COUNTIES OUTSIDE ATTESTED AREAS

	Total cattle on June 4th 1957 (provisional figures) ('000)	Number of registered attested herds	Total attested cattle ('000)	Percentage of total cattle
ENGLAND				
†Bedford	44	434	25	57
†Bucks	116	1,407	78	67
†Cambridge	32	283	19	59
Isle of Ely	20	112	6	30
Cheshire	245	3,282	153	62
†Cornwall	287	5,221	168	59
Derby	187	1,965	94	50
*†Devon	447	9,001	331	74
*Dorset	178	2,761	153	86
†Durham	128	2,098	82	64
†Essex	124	1,559	89	72
†Gloucester	205	2,631	140	68
†Hereford	156	3,448	125	80
†Hertford	60	767	47	78
Huntingdon	28	215	12	43
*Kent	127	2,198	102	80
Lancashire	285	5,356	214	75
Leicester	168	1,320	72	43
Lincoln-Holland	28	85	4	14
Kesteven	73	460	27	37
Lindsey	165	959	49	30
†Middlesex	7	88	4	58
†Norfolk	198	1,609	110	56
†Northampton	151	1,071	66	44
Soke of Peterboro'	8	49	4	50
Northumberland	215	1,783	103	48
Nottingham	112	1,019	51	46
†Oxford	109	1,440	84	77
Rutland	21	86	5	25
†Shropshire	301	4,176	196	65
*Somerset	343	6,367	274	80
Stafford	235	2,461	115	49
†Suffolk East	131	1,341	67	71
†Suffolk West		454	27	
*Sussex East	112	2,122	101	90
†Warwicks	149	1,511	82	55
*Wiltshire	217	2,889	191	88
†Worcester	108	1,161	55	51
Yorks East Riding	146	789	39	27
† North Riding	275	4,798	176	64
† West Riding	349	6,417	225	64
WALES				
†Denbigh	110	3,378	93	85
†Flint	63	1,183	40	63
†Glamorgan	80	1,901	48	60
†Monmouth	76	1,639	57	75
SCOTLAND				
†Aberdeen	253	4,849	175	69
†Angus	76	785	51	67
*†Banff	65	1,285	45	69
*Berwick	36	548	30	84
*East Lothian	18	340	16	88
†Kincardine	44	565	28	64
*Moray	35	739	30	87
*Perth	107	2,060	88	82
*Roxburgh	41	766	34	83

THERE WERE ALSO 3,025 SUPERVISED HERDS. THESE HERDS NEED ONLY ONE CLEAR TEST TO QUALIFY AS ATTESTED HERDS.

* Counties or parts of counties where free-testing facilities have been available since March 1st, 1956.

† Counties or parts of counties where free-testing facilities have been available since March 1st, 1957.

In part 2 the number of attested cattle and the derived percentage for counties in England and Wales are estimated figures.

Current Literature

ABSTRACT

Use of Diethylstilboestrol and other Hormone-like Substances for Livestock. BEESON, W. M. (1957). *N. Amer. Vet.* **38.** 265-8.

The author summarises the effects of female-like (diethylstilboestrol, hexoestrol, and dienoestrol) and male (testosterone) hormones on the development of beef cattle, sheep, and pigs.

Beef Cattle. Implanting low levels of diethylstilboestrol (24 to 30 mg.) at the base of the ears in steers will produce the same response in weight gain, feed efficiency, carcase quality, and general appearance as the oral feeding of 10 mg. of stilboestrol daily. Oral feeding requires 50 times more stilboestrol than implantation and costs 10 times as much. For steers in yards 36 mg. of diethylstilboestrol are used, and for steers on pasture 24 mg. of stilboestrol. One experiment proved that implantation is more effective in stimulating the gains of cattle fed on grain on pasture than oral feeding. The implants should be made at the beginning of the fattening period, and the effect usually lasts from 150 to 180 days. Feeding or subcutaneous implants of diethylstilboestrol will increase the gain by 15 to 25 per cent. and improve feed efficiency by about 10 per cent. Usually gains increase by 0.20 to 0.40 lb. daily. The female hormones usually increase the intake of food by about 3 per cent., but this is inconsistent. The response to stilboestrol is greater when cattle are fed adequate amounts of protein supplement than when they are not. Physical changes which occur in some cattle are an increase in the length of the teat, a weakening of the loins, and a prominence of the tailhead.

In most experiments the use of female hormones tends to depress the grade of the carcase or, at least, not to change it. Chemical analysis of the twelfth rib of cattle has indicated that feeding a female-like hormone to steers will decrease fat deposition by 2.5 per cent. and increase the protein level by 1 per cent. and the water level by 1.5 per cent. Feeding an antibiotic—either chlortetracycline or oxytetracycline—in combination with stilboestrol will tend to increase the deposition of fat and to counteract the depressing effect on the grade of the carcase. In tests in which 100 mg. chlortetracycline and 12 mg. of hexoestrol were fed for 103 days the daily gains were 2.03 lb. in control animals, 2.28 lb. with chlortetracycline, 2.5 lb. with hexoestrol, and 2.79 lb. with chlortetracycline and hexoestrol combined. Hormone-fed steers tend to shrink more in transit than control animals, and if this is not apparent, a lower dressing percentage is likely.

Approximately 50 per cent. of the stilboestrol ingested is excreted in the faeces and urine. Approximately 5 times more hormonal activity was noted in the faeces of cattle which were fed 10 mg. of stilboestrol daily than in steers used as controls. Cattle with implants of 36-mg. pellets of stilboestrol have little faecal oestrogenic activity.

Lambs. Although gains and feed efficiency are

(*Continued at the foot of col.* 1, *page* 1441)

News and Comment

THE BRITISH VETERINARY POULTRY ASSOCIATION

The fifth meeting of the Association was held in London at Unilever House on Friday, December 6th, 1957, and took the form of a symposium of 5 short papers. These were:—

1. "Riboflavin Deficiency in the Embryo in Association with Respiratory Disease in the Parent Flock" by J. D. Blaxland and B. S. Hanson; 2. "Some Aspects of Controlled Environments for Poultry" by W. P. Blount; 3. "Intestinal Coccidiosis in Ducklings" by S. F. Davies; 4. "Reflections on Renal Function in the Chicken" by M. H. Fussell; 5. "The Haemorrhagic Syndrome" by C. C. Wannop.

The papers stimulated a lively discussion after which members and guests enjoyed tea, kindly provided by Dr. Blount.

PRINTER'S PIE: AN APOLOGY

In last week's issue we had the misfortune to join the ranks of those journals (which include *The Times*) who have laid themselves open to merriment on the part of their readers through an untimely printer's error missed in proof reading. This unfortunately occurred to us with Mr. Cowie's letter which should have referred to "Pyometra in *Cats*." We ask Mr. Cowie to accept our apologies, and we would also point out to our readers that the dosage should read 1 to 2 *grains*.

It is some consolation (though not much) to be in good company in such a matter. Only last week-end those who have the privilege of serving the Queen were referred to in print as "ladies in wanting." A national newspaper once referred to a general as "this bottle-scarred veteran" and in its subsequent apology inserted "this battle-scared veteran." In a police court case a bookmaker's runner was described—not inaptly as it turned out—as a bookmaker's "ruiner." Perhaps the juiciest of these typographical morsels, however, appeared once in a local journal. It referred in its diary of events to the Parish Council holding a series of weekly "matings!"

Abstract.—*Concluded.*

significantly improved by the use of female-like hormones in feeder lambs, the quality of the carcase is depressed so that their use cannot be recommended.

Pigs. The feeding of 2 mg. diethylstilboestrol per hog daily has produced no beneficial effects on growth, feed efficiency, or carcase quality, but has produced such undesirable side effects as mammary stimulation in both gilts and hogs, and enlargement and congestion of the vulva in gilts. The feeding of methyltestosterone (male hormone) at levels of between 20 and 27 mg. per hog daily will produce more muscle and less fat, although gains and feed efficiency are not improved. At present their use is not practical and therefore not recommended.

J. O. L. K.

UNIVERSITY NEWS

Edinburgh

The undermentioned candidates have passed the Third Professional Examination (Part I) in Veterinary Pharmacology for the degree of Bachelor of Veterinary Medicine and Surgery.

Atkinson, William I.; McGregor, Donald S.; Mathieson, Andrew O.; Nicholson, Graeme G.

Glasgow

The following candidates passed in the final B.V.M.S. examination which was completed on December 10th, and they will graduate on Saturday, January 11th, 1958:—

Macleod, Norman S. M.; Peters, Ian T.; Pott, John M.; Roberts, David H.; Russell, Joseph S.; Thomson, James G.; Wills, Peter D.; Wilson, Robert.

R.C.V.S. OBITUARY

We record with regret the deaths of the following members of the profession:—

OFFORD, Edward Pegden Alec, 2, Clyde Villas, Hadley Green, Barnet, Herts. Captain late R.A.V.C. Graduated July 17th, 1909, at the Royal Veterinary College, London. Died December 9th, 1957, aged 72 years.

ROBSON, Herbert William, J.P., Glenroyd, Laurencekirk, Kincardineshire. Graduated July 17th, 1896, at the Royal Dick Veterinary College, Edinburgh. Died December 8th, 1957, aged 86 years.

HORMONES IN FEEDING-STUFFS

At a recent meeting of the Royal Highland and Agricultural Society of Scotland, it was decided to ask the Department of Agriculture for Scotland and the livestock departments of agricultural colleges to investigate the use of hormones in feeding-stuffs for fattening livestock. Breeders expressed anxiety about the possible dangers of the uncontrolled use of hormones, and it was stated that some farmers were adopting their use rather inadvisedly.

PERSONAL

Marriage

BRIDGES—BOUGHTON.—At Craignair Church, Dalbeattie, Kirkcudbrightshire, on December 7th, 1957, by The Rev. R. M. Boyd, M.A., Gordon Dennis Bridges, B.V.M.S., M.R.C.V.S., son of Mr. and Mrs. A. J. Bridges, to Barbara Amy Frances, daughter of Mr. and Mrs. F. W. Boughton, of Dalbeattie, Kirkcudbrightshire.

COMING EVENTS

January

8th, 9th and 10th (Wed., Thurs. and Fri.). R.C.V.S. Committee and Council Meetings.

17th (Fri.). Annual Buffet-Supper Dance of the Scottish Metropolitan Division, V.V.B.F. Ladies' Guild, 8 p.m.

February

7th (Fri.). Annual Ball of the Royal (Dick) School of Veterinary Studies in the Assembly Rooms, George Street, Edinburgh, 9 p.m.

B.V.A. COUNCIL AND COMMITTEE MEETINGS

Wednesday, January 22nd, 1958, at No. 7, Mansfield Street, W.1.

10.00 a.m. Veterinary State Medicine Committee.
11.30 a.m. Small-Animals Committee.
2.00 p.m. Farm Livestock Committee.

Thursday, January 23rd, 1958, at No. 7, Mansfield Street, W.1.

10.00 a.m. Parliamentary and Public Relations Committee.
11.30 a.m. Home Appointments Committee.
12.30 p.m. Finance Sub-committee.
2.15 p.m. General Purposes and Finance Committee.

Friday, January 24th, 1958, at the Connaught Rooms, W.C.2.

10 30 a.m. Council Meeting.

FOOT-AND-MOUTH DISEASE

The areas described in the schedules below are declared to be Infected Areas for the purpose of preventing the spread of foot-and-mouth disease:—

IN THE COUNTY OF KENT

The Borough of Tenterden.

In the Petty Sessional Division of Bearsted: The parishes of Hollingbourne, Frinsted, Lenham, Wichling, Otterden, Harrietsham, Broomfield, Leeds, Otham, Langley, Loose, Linton, Boughton Monchelsea, Chart Sutton, Sutton Valence, East Sutton, Ulcombe, Boughton Malherbe and Headcorn.

In the Petty Sessional Division of Ashford: The parishes of Charing, Westwell, Little Chart, Egerton, Smarden, Pluckley, Hothfield, Ashford, Great Chart, Bethersden, Woodchurch, Shadoxhurst, Kingsnorth, Wittersham, Stone cum Ebony, Appledore, Kenardington, and so much of the parishes of Warehorne, Orlestone and Ruckinge as lies to the north and west of the railway line running from Rye *via* Appledore to Ashford excluding the said railway.

In the Petty Sessional Division of Cranbrook and Tenterden: The parishes of Marden, Staplehurst, Frittenden, Biddenden, High Halden, Cranbrook, Rolvenden, Benenden, Newenden, Sandhurst, Hawkhurst, and so much of the parishes of Horsmonden and Goudhurst as lies to the north and east of the railway line running from Cranbrook *via* Goudhurst to Paddock Wood excluding the said railway.

In the Petty Sessional Division of Faversham: The parish of Stalisfield.

IN THE COUNTY OF WILTS

In the Petty Sessional Division of Tisbury and Mere: The parishes of Stourton with Gasper, Zeals, Mere, Sedgehill, West Knoyle and Kilmington and so much of the parish of East Knoyle as lies to the west of the main road (A.350) running from Shaftesbury *via* East Knoyle to Warminster, including the said road.

In the Petty Sessional Division of Warminster: The parishes of Maiden Bradley with Yarnfield and Kingston Deverill.

IN THE COUNTY OF DORSET

The Borough of Shaftesbury.
The Petty Sessional Division of Sturminster.

In the Petty Sessional Division of Shaftesbury: The parishes of Motcombe, East Stour, Stour Provost, Cann, Todber, Margaret Marsh, Melbury Abbas, Compton Abbas, East Orchard, West Orchard, Fontmell Magna, Sutton Waldron, Buckhorn Weston, Gillingham, Silton, Bourton and Iwerne Minster.

In the Petty Sessional Division of Blandford: The parishes of Iwerne Courtney and Shroton, Iwerne Steepleton, Stourpaine, Turnworth, Durweston, Winterborne Houghton and Winterborne Stickland.

In the Petty Sessional Division of Sherborne: The parishes of Bishop's Caundle and Holwell.

IN THE COUNTY OF SOMERSET

In the Petty Sessional Division of Wincanton: The parishes of Cucklington, Stoke Trister and Penselwood, so much of the parish of Charlton Musgrove as lies to the east of the road (B3081) running from Wincanton to Bruton, excluding the said road, and so much of the parish of Brewham as lies to the east of the railway running from Bruton to Westbury, excluding the said railway.

In the Petty Sessional Division of Frome: So much of the parish of Witham Friary as lies to the east of the railway running from Bruton to Westbury, excluding the said railway.

IN THE COUNTY OF EAST SUSSEX

The Petty Sessional Division of Frant.

In the Petty Sessional Division of East Grinstead: The parish of Hartfield and that part of the parish of Withyham as lies within the Petty Sessional Division.

In the Petty Sessional Division of Uckfield: The parishes of Buxted, Hadlow Down, Uckfield, Waldron, Framfield, Isfield, Little Horsted and East Hoathly.

In the Petty Sessional Division of Hailsham: The parishes of Heathfield, Warbleton and Horam and so much of the parish of Chiddingly as lies to the north of the main road running from Hailsham to Laughton, excluding the said road.

In the Petty Sessional Division of Battle: The parishes of Dallington and Brightling.

In the Petty Sessional Division of Burwash: The parishes of Burwash, Etchingham and Ticehurst.

IN THE COUNTY OF KENT

The Borough of Royal Tunbridge Wells.

In the Petty Sessional Division of Tonbridge: The parishes of Cowden, Speldhurst, Pembury, Brenchley, Bidborough, Southborough and Lamberhurst and so much of the parishes of Chiddingstone, Penshurst, Leigh, Tonbridge, Capel and Paddock Wood as lies to the south of the railway running from Redhill *via* Tonbridge to Marden, excluding the said railway.

In the Petty Sessional Division of Cranbrook and Tenterden: So much of the parishes of Horsmonden and Goudhurst as lies to the south and west of the railway running from Paddock Wood *via* Goudhurst to Cranbrook, excluding the said railway.

IN THE COUNTY OF EAST SUSSEX

In the Petty Sessional Division of East Grinstead: So much of the parishes of East Grinstead and Forest Row as lies to the north of the main road running from Three Bridges *via* East Grinstead to Hartfield, including the said road.

In the Petty Sessional Division of Uckfield: The parishes of Danehill, Maresfield and Fletching.

ADDRESSES OF DISEASE INFECTED PREMISES

The list given below indicates, first the county in which are situated the premises on which disease has been confirmed, followed by the postal address and date of outbreak.

Anthrax

Co. Durham. Travellers Rest Farm, Marwood Barnard Castle (Dec. 11).

Essex. Reedlands Farm, Holland Road, Little Clacton, Clacton-on-Sea (Dec. 10).

Hants. Causeway Farm, Hartley Witney, Basingstoke (Dec. 10).

Fowl Pest

Herts. Homefield, Bedmond Road, Bedmond, Watford (Dec. 13).

Lancs. 49, Town Road, Croston Preston; Wrennells Farm, Ridley Lane, Mawdsley, Ormskirk (Dec. 9); Debcross Farm, Station Road, Croston; Staleydene, Station Road, Little Hoole, Preston; Long Acre, Lancaster Lane, Leyland,

Preston; Hilltop, Lancaster Lane, Leyland, Preston; Gouldings Farm, Mawdsley, Ormskirk; Little Firs Farm, Leyland Lane, Leyland, Preston; 368, Dunkirk Lane, Leyland, Preston; 63, Dowbridge, Kirkham, Preston; 79, Buddle Lane, Walton-le-Dale Preston; Cowling Lane Farm, Cowling Lane, Leyland, Preston; Melrose, Pilling, Preston (Dec. 10); 350, Dunkirk Lane, Leyland, Preston; Rushmore, Shirley Lane, Longton, Preston; Snub Snape Farm, Altcar Farm, Leyland, Preston; Rydal Mount, Dowbridge, Kirkham (Dec. 11); Delmont, Lancaster Lane, Leyland, Preston; Common Bank Farm, Chorley; Clare Farm, Grange Lane, Hutton, Preston; Lane End Farm, Pompien Brow, Bretherton, Preston (Dec. 12); Auldene Nurseries, Southport Road, Ulnes Walton, Preston (Dec. 13); Tower House, Greenhalgh, Kirkham, Preston; Ivenof, Lancaster Lane, Leyland, Preston; Hawthorne Farm, Lancaster Lane, Leyland, Preston (Dec. 14).

Norfolk. Fernbank, Short Thorn Road, Stratton Strawless, Norwich; Woolpack Inn, Yaxham, East Dereham (Dec. 10); Home Leigh, Short Thorn Road, Stratton Strawless, Norwich (Dec. 11); Beech Farm, Yaxham, Dereham; Rosebank, Clint Green, Yaxham, East Dereham (Dec. 13).

Suffolk. The Green, Fakenham Magna, Bury St. Edmunds; Willow Hall Cottage, Troston, Bury St. Edmunds (Dec. 10).

Yorks. Greenacres, Sessay, Thirsk (Dec. 12).

Swine Fever

Anglesey. Brodawel, Llanfaes, Beaumaris (Dec. 10).

Derbys. Churchdale Farm, Ashford, Bakewell (Dec. 13).

Dorset. Piggeries, Middle Farm, Buckland Ripers, Weymouth (Dec. 9).

Essex. Corran House, Bramble Road, Thundersley, Hadleigh (Dec. 10); 50, Tilbury Road, Tilbury-Juxta-Clare, Great Yeldham, Halstead (Dec. 13).

Hants. New Farm, Winchester Road, Upham, Southampton (Dec. 9); Northside Farm, Bishops Sutton, Alresford (Dec. 11).

Leics. Shoby House Farm, Shoby, Melton Mowbray (Dec. 12).

Northants. The Elms, Denton, Peterborough (Dec. 13).

Notts. Piggeries off Radcliffe Road, Gamston, Radcliffe-on-Trent (Dec. 12).

Suffolk. Hall Farm, Redlingfield, Eye (Dec. 9); Riverside Farm, Needham Market, Ipswich (Dec. 10).

Worcs. Home Farm Powick (Dec. 10).

Yorks. Nidd View, Moor Monkton (Dec. 9); Hay Garth, Hull Road, Dunnington (Dec. 11); The Smallholding, Burton Salmon, Leeds (Dec. 13).

CORRESPONDENCE

The views expressed in letters addressed to the Editor represent the personal opinions of the writer only and their publication does not imply endorsement by the B.V.A.

LIVER CHANGES IN PARTURIENT CATTLE

Sir,—The liver lesion which sometimes occurs after the administration of dimidium bromide to cattle has been described and investigated experimentally (Plowright, Burdin & Thorold, 1952; Burdin & Plowright, 1952); and under field conditions, Wilde (1949) and Thorold and Plowright (1952) reported an increased incidence of dimidium bromide toxicity in parturient cattle. Other drugs bearing structural resemblance to dimidium bromide were administered to cattle (Ford & Wilson, 1955, unpublished observations) with the result that disturbances of liver function were noted in parturient cattle but not in non-pregnant animals which had received higher doses of the drugs under test. Moreover the toxic symptoms coincided more closely with the time of parturition than with a regular time interval after drug treatment as in the case following the administration of dimidium bromide in toxic doses to non-pregnant cattle.

These observations pointed to a need for the investigation of liver function in normal parturient cattle and with this end in view a number of tests were carried out on shorthorn cattle maintained at this Institute. Serum alkaline phosphatase and serum bilirubin levels were determined at intervals before and after parturition and liver biopsy material, fixed immediately in neutral formol-saline, was examined for general morphology and for histological evidence of fat in hepatic cells. A liver homogenate was also examined for alkaline phosphatase activity.

From the various results two features are prominent. One is the well-marked appearance of stainable fat in the hepatic cells within a short time of parturition followed by the disappearance of this material from the hepatic cells seen in biopsy material taken during lactation.

Wolterstorff (1921) had previously described fatty infiltration of hepatic cells in pregnant cattle but in working with slaughter-house material he had not been able to follow the gradual disappearance of the stainable fat during early lactation.

The second alteration in liver function noted during these observations was a rise in the alkaline phosphatase activity of liver homogenates around the time of parturition, followed by a gradual fall, as lactation proceeds, to values in the region of those obtained during pregnancy. An analysis of these observations is given in Tables I and II.

TABLE I
ALKALINE PHOSPHATASE ACTIVITY OF LIVER HOMOGENATES (K.A. UNITS/100 G. LIVER)

Group	1	2	3	4	5	6
Days from parturition	60–41	40–21	20–1	1–20	21–40	41–60
Number of observations	9	12	13	20	13	6
Mean value	366	382	479	576	404	396
Standard error	±63	±44	±41	±37	±67	±37

TABLE II
COMPARISON OF GROUPS

Groups compared	1 & 4	2 & 4	3 & 4	4 & 5	4 & 6
Degrees of freedom	27	30	31	31	24
"t"	3·04	3·34	1·73	2·43	2·56
Probability	<·01	<·01	<·1	<·05	<·02

A number of other general metabolic changes in normal parturient cattle have already been described, for example temporary hyperglycaemia, hypocalcaemia and hypophosphataemia (Godden & Allcroft, 1932), leucocyte "crossover" (Kerr, Robertson & McGirr, 1951) and increased plasma 17-hydroxycorticosteroids (Paterson, 1957), and the demonstration of the transient appearance of stainable hepatic fat together with the rise in hepatic alkaline phosphatase activity adds support to the view that parturition is a period of profound metabolic changes in the cow.

The significance of these two changes and of other metabolic disturbances at the time of parturition is

being further investigated with particular reference to the onset at this time of such diseases as acetonaemia.

Yours faithfully,
E. J. H. FORD.

A.R.C. Institute of Animal Physiology,
Babraham Hall,
Babraham,
Cambridge.

November 20th, 1957.

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The Experimental Production of Scrapie in Goats

Sir,—In a previous communication (Pattison, 1957)* it was reported briefly that scrapie had been transmitted to goats by intracerebral inoculation of brain tissue from a scrapie-affected sheep. The disease has now been passaged in series through three groups of goats by intracerebral inoculation and has been passed back again to sheep from goats. Thus, of 20 South Scotland Cheviot sheep inoculated intracerebrally with brain tissue from scrapie-affected goats, 8 have developed classical symptoms of scrapie.

A feature of distinct interest is the fact that intracerebral inoculation with brain tissue, or with cerebrospinal fluid, from scrapie-affected animals, has produced the disease in all of 67 goats that have been under observation for a sufficient length of time for the disease to develop. The distribution of these cases according to the period between inoculation and appearance of the disease has been:—

Number of months between inoculation and the appearance of clinical symptoms of scrapie.							
7	8	9	10	11	12	13	14
Distribution of 67 cases.							
1	10	7	8	4	5	10	6

Number of months between inoculation and the appearance of clinical symptoms of scrapie.							
15	16	17	18	19	20	21	22
Distribution of 67 cases.							
1	2	2	3	2	2	2	2

Control intracerebral inoculations of 11 normal goats with brain emulsion from a normal sheep were made 18 months ago, and, to date, these animals have shown no evidence of disease.

In due course, it is hoped to publish a detailed account of experimental scrapie in the goat, but since the disease takes so long to appear after inoculation it will be some considerable time before all the necessary material is available for publication. If this 100 per cent. "take" following intracerebral inoculation of goats continues to hold good, it will be helpful in the investigation of scrapie which has been hampered not only by the long interval between inoculation and the appearance of symptoms, but even more by the low rate of experimental infection (at best about 60 per cent.) encountered in sheep.

Yours faithfully,
W. S. GORDON.
I. H. PATTISON.

Agricultural Research Council Field Station,
Compton,
Berks.

December 11th, 1957.

* *Lancet* (1957). Jan. 12th, p.104.

Use of Synthetic Oestrogens for Fattening Cattle

Sir,—With reference to recent reports of discussions on possible dangers to man resulting from the use of synthetic oestrogens for fattening cattle, American experience is that the nation's meat eaters have little to fear. Current U.S. production of supplemented feed amounts to 7,000,000 to 9,000,000 tons per annum, about 25 per cent. of the total feed market, and many different drugs are added to it. How much stilboestrol gets into feed is not readily ascertainable but it is probable that more than half of the beef cattle of the U.S.A. are fed some amount, and that this is of the order of 10 to 11 mg. per head per day.

The employment of synthetic oestrogens in poultry was previously the cause of much agitation and questioning and was settled in favour of the drugs. Now their contemplated use in cattle in Britain is giving rise to the same kind of reaction. All of the very wide U.S. experience indicates that no risk is involved for consumers, and there is ample evidence that these products have very desirable effects both on quality of end product and cost of production.

Yours faithfully,
DAVID D. OGILVIE.

48, Vanderbilt Road,
Manhasset, L.I.,
New York,
U.S.A.

December 12th, 1957.

Partners and Assistants

Sir,—A rather unusually phrased advertisement in the Partnership section of the advertisements in this week's (December 7th) VETERINARY RECORD prompts me to ask, if I may, whether any reader can explain what a salaried partner is and how this post differs from that of an assistant?

One is tempted to think that the difference may be of the Smith-Smythe order.

Yours faithfully,
ROBERT S. MOIR.

St. Margarets,
Eaglesfield,
Lockerbie,
Dumfriesshire.

December 10th, 1957.